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### ▶ To cite this version:

Søren R. Paludan, Thomas Pradeu, Seth L. Masters, Trine H. Mogensen. Constitutive immune mechanisms: mediators of host defence and immune regulation. Nature Reviews Immunology, 2020, 10.1038/s41577-020-0391-5. hal-02951558

## HAL Id: hal-02951558 https://hal.science/hal-02951558v1

Submitted on 28 Sep 2020

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#### Constitutive immune mechanisms: mediators of host defence and immune regulation

Søren R. Paludan, Thomas Pradeu, Seth L. Masters & Trine H. Mogensen

Nature Reviews Immunology (2020)

#### Abstract

The immune system enables organisms to combat infections and to eliminate endogenous challenges. Immune responses can be evoked through diverse inducible pathways. However, various constitutive mechanisms are also required for immunocompetence. The inducible responses of pattern recognition receptors of the innate immune system and antigen-specific receptors of the adaptive immune system are highly effective, but they also have the potential to cause extensive immunopathology and tissue damage, as seen in many infectious and autoinflammatory diseases. By contrast, constitutive innate immune mechanisms, including restriction factors, basal autophagy and proteasomal degradation, tend to limit immune responses, with loss-of-function mutations in these pathways leading to inflammation. Although they function through a broad and heterogeneous set of mechanisms, the constitutive immune responses all function as early barriers to infection and aim to minimize any disruption of homeostasis. Supported by recent human and mouse data, in this Review we compare and contrast the inducible and constitutive mechanisms of immunosurveillance.

#### Introduction

A major challenge for living organisms is to maintain homeostasis in response to changes in external and internal environments. These include alterations in nutrient and water supplies, physical stress, temperature changes, physiological stress, infections and malignancies<sup>1</sup>. Through billions of years of evolution, the forms of life and biological processes that cope with these challenges in the most successful way have been selected. One challenge that all organisms have to deal with is the elimination of microorganisms and of abnormal or damaged cellular material. The ideal immune response would eliminate the potential threat and re-establish homeostasis without causing excessive damage to healthy cells and tissues. However, immune responses to infections are often disruptive and can cause marked tissue damage<sup>2,3</sup>. Such responses are evolutionarily advantageous when the benefit of eliminating the challenge outweighs the risk of associated tissue damage and the requirement for regeneration. However, for potential challenges that occur frequently but rarely develop into serious homeostasis-altering threats, it is not desirable to mount systemic or potentially disruptive immune responses. In addition, vigorous immune responses are not desirable in organs and tissues that are particularly sensitive to immune-mediated damage, such as the brain. Therefore, the ideal immune response has checks and balances, which allow the organism to modulate the magnitude and duration of the response according to the nature of the threat caused by the challenge.

The mammalian immune system, as we understand it today, is induced mainly by two types of receptor systems, the germline-encoded <u>pattern recognition receptors</u> (PRRs), which initiate innate immune responses, and the antigen-specific receptors generated through gene rearrangement after antigen encounter, which initiate adaptive immune responses<sup>4,5,6</sup>. The immune responses induced by PRRs, such as Toll-like receptors (TLRs), interact with those induced by antigen-specific receptors; this interaction is notably represented by dendritic

cells, which rely on PRR-driven cues to initiate dendritic cell maturation for the stimulation of lymphocytes through antigen-specific receptors<sup>5</sup>. However, the research literature contains numerous reports of host defence activities that occur independently of both PRR-based immunity and antigen-specific receptors<sup>7,8,9,10</sup>, and emerging evidence suggests that several of these mechanisms have non-redundant roles in host defence in humans<sup>11,12</sup>. Here we review the literature on this topic by focusing on <u>constitutive immune mechanisms</u>. On the basis of this analysis, and by integrating concepts previously reviewed<sup>13</sup>, we propose that this constitutive layer of innate immunity exerts early host defence activities through specific molecular mechanisms and at the same time limits PRR activation as a specific feature.

#### Constitutive and inducible mechanisms

The innate immune system uses both constitutive and <u>inducible mechanisms</u> to eliminate infections and damaged self to maintain homeostasis (Fig. <u>1</u>). Although the constitutive mechanisms have the advantage of providing an immediate response to a danger signal, they lack the potential to amplify the response. In addition, constitutive mechanisms consume energy to remain operative, and there are hence limits to how many of these can be maintained in any one organism. By contrast, inducible mechanisms such as those mediated through PRRs, as well as antigen-specific receptors, are activated only in response to stimuli and have the ability to amplify signals many times. Hence, inducible mechanisms can give rise to very strong and efficient immune responses, but can also lead to excess inflammation and immunopathology. Given their amplification potential, inducible immune mechanisms require tight control and negative regulatory systems.

#### Fig. 1: Constitutive innate immune responses versus inducible immune responses.



Illustration of how constitutive and inducible immune responses vary over time during the course of a generalized infection, and their impact on host defence, energy consumption and host fitness. In the case of a sterilizing and resolving immune response, the additional energy consumption required by the inducible immune response is balanced by the re-establishment of homeostasis. By contrast, in the case of an immunopathological response, the energy that is consumed to mount an inducible response does not benefit the host and instead leads to tissue damage and disruption of homeostasis.

The constitutive immune mechanisms can be divided into the chemical and physical barriers of the body, such as skin, saliva, stomach acid and urine flow, which are not the focus of this Review, and various molecularly defined mechanisms that control microbial infection and/or replication<sup>1</sup>. Although these mechanisms have been known for many years, they have generally been considered to have only minor roles in the immune system, and evidence has been lacking as to their specific, non-redundant functions in host defence. Consequently, they

have not received much attention in front-line immunology research. Here we discuss the constitutive innate immune responses in comparison with the better-described inducible innate responses triggered by PRRs. In addition, we present evidence suggesting that efficient action of constitutive innate immune mechanisms leads to both antimicrobial activity and mitigation of PRR-driven activities (Fig.  $\underline{2}$ ).



Fig. 2: Constitutive innate immune responses negatively regulate inducible immune responses.

**a** | Constitutive innate immune mechanisms eliminate pathogens during the initial stages of an infection, which prevents the accumulation of pathogen-associated molecular patterns (PAMPs) that would otherwise activate an inducible immune response through pattern recognition receptors (PRRs). In addition, many of the constitutive mechanisms are known to directly downregulate PAMP signalling through PRRs. Both of these effects limit PRR-induced expression of type I interferon and IL-1 $\beta$ . **b** | The relationship between the different proposed layers of the immune response. A first layer of defence is exerted by physical and chemical barriers. Constitutive innate immune mechanisms function as soon as a danger signal is detected and eliminate harmful microorganisms and host molecules by specific non-inflammatory mechanisms that operate independently of PRRs. This prevents establishment of the infection and accumulation of PAMPs, thus limiting the activation of PRR-based inducible innate immune responses. If PRR-based immunity is activated, owing to the level of PAMPs exceeding a certain threshold, this leads to inflammation and promotes activation of the adaptive immune response mediated by T cells and antibodies. IRF, interferon regulatory factor.

#### PRR-activated inducible innate immune responses

PRRs detect pathogen-associated molecular patterns (PAMPs), microorganism-associated molecular patterns<sup>14</sup>, host-derived danger-associated molecular patterns<sup>15</sup> and molecular signatures associated with homeostasis-altering molecular processes<sup>16</sup>. These molecular patterns activate PRR signalling, which ultimately leads to the transcription of antimicrobial and proinflammatory genes. Downstream activities of PRR signalling include the production of type I interferon (interferon- $\alpha$  (IFN $\alpha$ ) and IFN $\beta$ ), IL-1 $\beta$  and tumour necrosis factor (TNF). These cytokines, in turn, activate antimicrobial and proinflammatory activities, as well as the maturation of antigen-specific adaptive immune responses<sup>17,18</sup>. PRR-based immune responses can be highly potent, and numerous inflammatory diseases are driven by excessive PRR signalling pathways<sup>2,19,20</sup> (Box <u>1</u>). However, the nature of PRR-based immunity is influenced by many factors, and it is worth mentioning that the gut microbiota and chronic viral

infections can induce PRR-based, host-beneficial responses that tend towards tolerance rather than inflammation<sup>21,22</sup>. Nevertheless, given the potency of PRR-based immunity, full activation of PRR-driven immune responses each time a microorganism is encountered may not be beneficial for an organism in the longer term. Moreover, it is essential to control the activation and duration of PRR signalling-induced activities. This is achieved through multiple mechanisms, including two-step procedures for full PRR activation $\frac{23,24}{2}$ , the requirement for a threshold PAMP concentration to achieve PRR activation<sup>25,26,27,28</sup>, amplification loops from initial low responses<sup>29</sup> and numerous negative-feedback mechanisms $\frac{30}{2}$ . One way in which the activation of PRR signalling in response to very low levels of PAMPs is avoided at the molecular level is through supramolecular organizing centres. These are higher-order signalling complexes at specific subcellular locations that rely on amplification mechanisms to achieve full activation, thus preventing signalling by subthreshold levels of PAMPs but amplifying signalling by superthreshold levels of PAMPs<sup>29</sup>. The double-edged sword-like nature of PRR-induced immune responses in terms of their roles in both protection and disease is also supported by evolutionary evidence. This includes the recurring loss of 2'-5'-oligoadenylate synthase 1 (OAS1) in primates<sup>31</sup>. OAS1 is an interferon-inducible protein that is associated with both antiviral and pathological activities<sup>32,33</sup>

#### Constitutive innate immune mechanisms

Constitutive innate immune mechanisms respond to microbial activities, cellular stress and metabolic alterations by inducing antimicrobial effector functions. As there is most evidence for constitutive innate immune mechanisms that exert antiviral and antibacterial activities, these are the focus of this Review (Fig. <u>3</u>). A large range of constitutive mechanisms of innate immunity have been identified, including restriction factors, antimicrobial peptides, basal autophagy and proteasomal degradation (Box <u>2</u>; Table <u>1</u>). Here we divide these mechanisms into two classes: those that target specific steps in microbial replication cycles, such as restriction factors<sup>34,35</sup>, and those that lead to degenerative processes, such as autophagy<sup>9,36</sup>. The constitutive mechanisms that target specific steps in microbial replication function by blocking molecularly defined events that are essential for the replication of specific microorganisms but are dispensable for cellular fitness. By contrast, those mechanisms that operate through degenerative programmes target microbial or altered host molecules for recycling or degradation. The modes of action of representative examples from each of these mechanistic classes are described in the following sections.

# Fig. 3: Overview of the regulation of microbial replication by constitutive innate immune mechanisms.

#### a Viral infection



**a** | Constitutive innate immune mechanisms and viral infection. The accumulation of specific viral molecular structures (such as double-stranded RNA (dsRNA) or capsids) and cellular stress responses (such as autophagy) activate constitutive-latent mechanisms with direct antiviral activity, independently of pattern recognition receptors. Some of the antiviral effector functions target microbial replication by blocking specific steps in the replication cycles of viruses; these effectors include soluble lectins, antimicrobial peptides, restriction factors, RNA interference (RNAi) and metabolites. Other antiviral effectors of the constitutive response function through the degradation of virus particles; these include nucleases such as TREX1, which degrades viral DNA in the cytoplasm, and RNase L, which degrades viral RNA, as well as autophagy and proteasomal degradation. Viruses can be targeted for proteasomal degradation by the ubiquitin E3 ligase TRIM21, which binds to antibody-attached viral capsids. **b** | Constitutive innate immune mechanisms and bacterial infection. The presence of bacteria changes the local microenvironment, for example through the accumulation of hydrophobic and charged bacterial surfaces or alteration of cellular metabolism. This activates antibacterial activities independently of pattern recognition receptors, including inactivation by soluble lectins and antimicrobial peptides, nutritional depletion by natural resistance-associated macrophage protein 1 (NRAMP1) and lactoferrin, and bacterial degradation by phagocytosis and basal autophagy. dsDNA, double-stranded DNA; RISC, RNA-induced silencing complex; ROS, reactive oxygen species; viRNA, virusderived small interfering RNA.

#### Table 1 Constitutive immune mechanisms in host defence

Given the ability of constitutive immune mechanisms to exert antimicrobial activity, one consequence of their successful action is decreased levels of PAMPs (Fig. 2a). This, in turn, limits PRR activation and the downstream inflammatory response (Fig. 2b). Thus, constitutive immune mechanisms equip cells and tissues with a layer of defence that can fight infections immediately and hence potentially limit the requirement for inducible immune responses, such as type I interferon, IL-1 $\beta$  and other proinflammatory cytokines.

#### **Targeting microbial replication**

Direct inhibition of microbial replication is executed by molecules that interfere with specific steps in the replication cycle of a given microorganism. There are at least six mechanisms of action in this category: restriction factors that directly block a specific replication step; restriction factors that deplete molecules essential for replication; <u>RNA interference</u> (RNAi); antimicrobial peptides; soluble lectins; and metabolite-mediated inhibition of microbial replication (Table <u>1</u>).

#### **Restrictions factors**

Restriction factors are antiviral proteins that target viral replication. Extensive studies, particularly of HIV-1 and herpesviruses<sup>37,38</sup>, have led to the identification of numerous restriction factors that together target nearly all steps in the viral replication cycle (Fig. <u>4a</u>). For example, APOBEC3 proteins belong to the family of cytidine deaminases, which catalyse the deamination of cytidine to uridine in single-stranded DNA, thus introducing potentially deleterious mutations into the HIV-1 genome<sup>39</sup>. Likewise, tetherin is a membrane-bound protein that prevents the release of progeny HIV-1 particles from the cell surface<sup>40</sup>. These two mechanisms provide examples of direct blockade of specific steps in the replication cycle. By contrast, SAM domain and HD domain-containing protein 1 (SAMHD1) blocks HIV-1

replication indirectly, by converting deoxynucleoside triphosphates into inorganic phosphate and 2'-deoxynucleoside, thus depleting essential building blocks for HIV-1 reverse transcription  $\frac{34,41}{1}$ . The aforementioned restriction factors work in the plasma membrane or in the cytoplasm. However, many DNA viruses, including herpesviruses, replicate in the nucleus, where they are also targeted by numerous restriction factors. These include nuclear domain 10 bodies (ND10 bodies) and IFNy-inducible protein 16 (IFI16), which operate by different mechanisms to epigenetically silence viral genomes<sup>35,42</sup>. The herpesvirus DNA rapidly associates with ND10 bodies, which restrict viral gene expression by promoting processes that lead to the formation of nucleosome-like structures  $\frac{42}{2}$ . IFI16 restricts viral replication in the nucleus mainly by interfering directly with transcription $\frac{35}{2}$ . New evidence suggests that this involves the ability of IFI16 to form DNA filaments, which reduces recruitment of RNA polymerase II (ref.<sup>43</sup>), but also leads to recruitment of ND10 bodies, thus indicating that these two restriction systems might interact. The restriction factors discussed here are all constitutively expressed, although the expression of many of them is further increased by interferons<sup>35,44,45</sup>. Tonic type I interferon signalling or constitutive activity of interferon regulatory factor 1 (IRF1) drives the basal expression of many constitutive restriction factors<sup>8,46,47</sup>.

# Fig. 4: Constitutive control of microbial replication by restriction factors and autophagy.



**a** | Restriction factors that control herpesvirus and retrovirus infections, including their targets in the viral replication cycle. Restriction factors interfere with viral replication by either blocking a specific and essential step in the viral replication cycle (for example, viral gene transcription or release of progeny virus) or depletion of factors that are essential for replication (such as deoxynucleoside triphosphates). **b** | Blockade of viral and bacterial replication by autophagy. Various ubiquitin E3 ligases (such as SMURF1, LRSAM1 and TRIM23) and ubiquitin-binding proteins (such as p62, optineurin and NDP52) have been identified to conjugate ubiquitin to microbial surfaces, which targets them for loading into autophagosomes. Also, cytosolic exposure of glycans by pathogen-damaged vesicles can be recognized by galectin 8 for targeting to autophagosomes. APOBEC3, apolipoprotein B mRNA-editing complex 3; BST2, bone marrow stromal antigen 2 (also known as tetherin);

DBR1, RNA lariat debranching enzyme 1; IFI16, interferon-γ-inducible protein 16; IFITM, interferon-induced transmembrane protein; MTOC, microtubule-organizing centre; ND10, nuclear domain 10; SAMHD1, SAM domain and HD domain-containing protein 1; SIRT6, sirtuin 6; SNORA31, small nucleolar RNA, H/ACA box 31.

#### **RNA interference**

RNAi is another constitutive immune mechanism that directly controls viral replication. RNAi involves the processing of double-stranded RNA molecules by members of the Dicer nuclease family to 20–25-bp fragments, thus leading to the formation of the RNA-induced silencing complex (RISC), which blocks gene expression or translation through binding to target mRNAs<sup>48</sup>. The ability of RNAi to directly block viral replication was first shown in plants<sup>49</sup> and was later also shown in insects and worms<sup>50,51,52</sup>. For example, *Caenorhabditis elegans* and *Drosophila melanogaster* infected with Flock House virus activate antiviral defence mechanisms that depend on Dicer<sup>51,53</sup>. This constitutive immune mechanism might have a more important role in lower organisms, but as some mammalian viruses do target the RNAi system, there may be a subdominant role for this primordial antiviral system in host defence in more evolved organisms<sup>54</sup>. For example, Ebola virus VP35 and VP30 proteins interact with Dicer cofactors, and the hepatitis C virus core protein directly associates with Dicer<sup>55,56</sup>.

#### **Antimicrobial peptides**

Antimicrobial peptides, including defensins and cathelicidins, contribute to the first line of defence against bacteria in the skin and at mucosal surfaces. They work by binding directly to bacterial membranes, thus perturbing membrane integrity and inhibiting microbial growth<sup>57,58,59,60</sup>. These peptides are rich in both cationic and hydrophobic amino acids, and generally form amphiphilic helical structures, although this may not be the case for all antimicrobial peptides<sup>61</sup>. This enables the peptides to interact with negatively charged bacterial surfaces through electrostatic interactions, thus triggering disruption of the bacterial membranes by pore-forming or non-pore-forming mechanisms<sup>62</sup>. Many antimicrobial peptides, such as  $\beta$ -defensin 1, are constitutively expressed on epithelial surfaces, thus providing immediate antimicrobial action on infection $\frac{63}{2}$ . This is illustrated by the increased susceptibility to a broad range of bacterial infections in mice lacking cathelicidin antimicrobial peptide  $(CAMP)^{59,64}$ . Beyond their role in antibacterial defence, there is also evidence that antimicrobial peptides can disrupt viral particles, thus exerting antiviral activity<sup>65,66</sup>. Similarly to the restriction factors, many antimicrobial peptides are expressed in both constitutive and inducible manners. This illustrates the general principle that different branches of the immune system can use overlapping effector functions (Box 2).

#### Soluble lectins

Many microorganisms have extensive and more complex glycan patterns than mammalian cells, and these sugars can therefore be used as a means to distinguish self from non-self. There are four classes of soluble lectins carrying out this function, namely collectins, ficolins, galectins and pentraxins<sup>67</sup>. On recognition of non-self glycans, soluble lectins can exert host defence activities indirectly through complement activation and opsonization, as discussed later, or directly through aggregation and neutralization. For example, the collectin surfactant protein D (SP-D) has been reported to bind directly to highly glycosylated viruses such as HIV-1 and influenza A virus and neutralize their infectivity<sup>68,69</sup>. Similarly, pentraxin 3

directly binds influenza A virus particles and neutralizes virus infectivity<sup>70</sup>. Importantly, SP-D-deficient mice have impaired clearance of influenza A virus and increased production of proinflammatory cytokines in response to viral challenge<sup>71</sup>. In addition to viruses, SP-D also binds and agglutinates *Streptococcus pneumoniae*<sup>72</sup>, thus suggesting that soluble lectins might also have a role in the immediate inactivation of bacteria.

#### Metabolite-mediated inhibition

A final example of constitutive immune mechanisms that directly interfere with microbial growth is provided by metabolites that block pathogen replication, and perhaps the best example of which is lactate<sup>73,74</sup>. Many viral infections are characterized by a shift of host cellular metabolism to <u>aerobic glycolysis</u>, which leads to the production of lactate<sup>25,76</sup>. Viral infections also induce fatty acid synthesis and intermediate molecules in these pathways. These include palmitic acid and oleic acid, which have been shown to have antiviral activity<sup>77,78</sup>. The mechanisms by which lactate and other metabolites block viral replication remain to be determined, but the antiviral activity of lactate illustrates a general principle that select molecules accumulating during alterations of cellular homeostasis can interfere with microbial replication.

A second form of metabolite-dependent constitutive host defence is mediated through nutritional depletion and starvation of pathogens. For example, natural resistance-associated macrophage protein 1 (NRAMP1; also known as SLC11A1) is a metal ion transporter that transports divalent cations from vacuoles into the cytoplasm, hence depleting factors from vacuoles that are essential for the growth of intracellular pathogens<sup>79</sup>. The gene encoding NRAMP1 was shown to contribute to defence against, for example, *Mycobacterium tuberculosis*, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Leishmania donovani*<sup>80,81</sup>, which was later shown to be mediated by the reduction of metal ion concentrations inside microorganism-containing vacuoles<sup>82</sup>. A second example of nutritional depletion is provided by lactoferrin, which is present in various secretory fluids. Lactoferrin is a highly cationic molecule that shows antimicrobial activity, in part, by binding and sequestering iron from pathogenic microorganisms<sup>83</sup>. Lactoferrin contributes to host defence in a non-redundant manner, as lactoferrin-deficient mice have increased susceptibility to *Streptococcus mutans*-induced dental caries, for example<sup>84</sup>.

#### **Degenerative mechanisms**

The second class of constitutive innate immune mechanisms functions through the degradation of danger molecules and elimination of unwanted cells. This class of mechanisms includes autophagy, phagocytosis, proteasomal degradation and nucleases (Table <u>1</u>). Collectively, degenerative programmes function to continually limit danger signals, allowing for the rapid elimination of unwanted molecules without the activation of energy-consuming amplificative induced immune responses.

#### Autophagy and phagocytosis

Autophagy and phagocytosis execute the digestion of intracellular and extracellular microorganisms, respectively, through membrane encapsulation followed by chemical and enzymatic degradation<sup>85,86</sup>. Pathogens are shunted into these pathways through the recognition of polyubiquitin chains or glycans inside damaged vacuoles in the case of autophagy<sup>9,87</sup>, and through complement coating of microorganisms in the case of

phagocytosis<sup>88</sup>. In the case of autophagy, a large number of ubiquitin E3 ligases have been identified that coat viral and bacterial surfaces with ubiquitin<sup>9,89,90,91,92</sup>, thus targeting microorganisms for loading into autophagosomes through interaction with the autophagosome-associated protein LC3 (also known as MAP1ALC3)<sup>85</sup> (Fig. 4b). This targeting mechanism involves E3 ligases, including SMURF1 and LRSAM1 (refs<sup>91,92</sup>), as well as the ubiquitin-binding selective autophagy receptors p62 (also known as SQSTM1), optineurin and NDP52 (also known as CALCOCO2)<sup>9,89,93</sup>. An alternative mechanism for sensing of vesicle-damaging pathogens has been identified that involves damaged vesicles exposing glycans in the cytoplasm for sensing by galactin 8, which links to autophagy via NDP52 (ref.<sup>87</sup>). This triggers phagophore formation in the vicinity of cytosolic bacteria<sup>94</sup>. Autophagy has important roles in the control of infection. For example, defective autophagy leads to increased susceptibility to infection with Sindbis virus in mice<sup>89</sup>. In addition, stimulation of autophagy in primary human macrophages mediated protection against M. *tuberculosis* infection<sup>95,96</sup>. However, mice defective in autophagy do not have impaired antimycobacterial defence in vivo, which indicates that the precise role of autophagy requires further investigation<sup>97</sup>. Third, herpes simplex virus type 1 specifically interferes with autophagy, which is essential for neuropathogenicity of the virus $\frac{36}{2}$ .

Complement-mediated phagocytosis involves specific recognition of complement components bound to the surface of microorganisms by the corresponding complement receptors on phagocytes. Activation of the complement system, for example after sensing of glycans by the lectin pathway, leads to the formation of C3 convertase, eventually generating C3b, which binds to complement receptors, thus inducing phagocytosis<sup>98</sup>. Mice devoid of the lectin-based complement pathway have increased susceptibility to *Staphylococcus aureus* infection and impaired bacterial phagocytosis<sup>99</sup>. Furthermore, several bacteria, including *Streptococcus pyogenes*, inhibit complement-mediated phagocytosis<sup>100</sup>.

A third degenerative mechanism for the degradation of membrane-encapsulated extracellular material is LC3-associated phagocytosis (LAP), which uses components from both the phagocytosis and autophagy pathways<sup>101</sup>. LAP is involved in the clearance of extracellular pathogens and dead cells<sup>102</sup>, and LAP-deficient mice fail to clear *Aspergillus fumigatus* infection<sup>103</sup>. Thus, autophagy, phagocytosis and LAP are important systems for immediate host defence.

#### **Proteasomal degradation**

The proteasome is a cytoplasmic protein complex that degrades proteins by proteolysis<sup>104</sup>. Proteins to be degraded are tagged by K48-linked polyubiquitylation, attracted to the proteasome, unfolded into polypeptides and then degraded<sup>104</sup>. The proteasomal degradation pathway also contributes to immediate defence against infecting pathogens. For example, viruses can be detected by the ubiquitin E3 ligase TRIM21 through binding to antibody-bound viral capsids, which links to downstream proteasomal degradation<sup>105</sup>. This process is involved in the elimination of infecting viral capsids from the cytoplasm and contributes to antiviral defence<sup>105,106,107</sup>. Other studies have shown that the viral RNA-dependent RNA polymerase of turnip yellow mosaic virus is degraded by the ubiquitin–proteasome pathway to control infection<sup>108</sup>. Proteasome activity also contributes to defence against many bacterial infections, including *Yersinia* spp. infections<sup>109</sup>, and the ubiquitin–proteasome pathway is targeted by many viruses and bacteria to promote replication<sup>110,111,112,113,114</sup>. For example, the human cytomegalovirus protein pUL25 inhibits proteasomal degradation of another viral protein, pUL26, to sustain the activity of a pUL26-

mediated immune evasion mechanism<sup>114</sup>. Collectively, these examples show that the conserved proteasome pathway is part of the constitutive immune defence repertoire.

#### Nucleases

The cytoplasm contains RNAses and DNAses that eliminate unwanted nucleic acid species, including viral nucleic acids, and these enzymes can thereby contribute to sterilization of the cytoplasm. RNase L is a latent cytoplasmic exoribonuclease that is activated by 2'-5' oligoadenylates produced by OASs<sup>115</sup>. Although OASs are highly interferon inducible, they are also expressed at a basal level and hence induce basal RNase L activity<sup>116</sup>. Importantly, this activity has been suggested to contribute to basal restriction of coronaviruses in myeloid cells, and hence to protect other cell types from infection<sup>117</sup>. TREX1 is a cytoplasmic exodeoxyribonuclease that eliminates DNA from the cytoplasm. Very few microorganisms have free DNA as part of their productive replication cycle, but exogenous and endogenous retroviruses have a cytoplasmic DNA step that is sensitive to degradation by TREX1. Consequently, *Trex1<sup>-/-</sup>* mice have increased levels of endogenous retroviral DNA in the cytoplasm<sup>118</sup>, which indicates that TREX1 has a role in limiting retroviral infection and hence maintaining genome integrity.

#### Limiting inflammatory responses

Immune responses induced by PRRs and by antigen-specific receptors are often highly potent and sterilizing. However, they may also be relatively disruptive and can be associated with tissue damage and the requirement for significant tissue repair and energy consumption<sup>119</sup>. Many of the constitutive immune mechanisms discussed here not only interfere with microbial replication but also have negative effects on PRR activity (Table <u>1</u>). This raises the possibility that an overarching function of the constitutive immune mechanisms is to both eliminate danger and limit the use of PRR-driven activities. At the mechanistic level, this immunoregulatory function of the constitutive mechanisms can be exerted in two qualitatively different ways. The first is through the direct effect of their antimicrobial activity on decreasing levels of PAMPs. The second is through specific inhibition of PRR signalling.

#### **Reduction of PAMP levels**

Many studies have shown that PRR activation requires PAMP levels to be above a certain threshold<sup>25,26,27,28</sup>. Above this threshold, PRRs are activated in a concentration-dependent manner until saturation is reached. Therefore, constitutive immune mechanisms that reduce PAMP levels will limit or even prevent PRR activation (Fig. 2a). For example, mice deficient in the restriction factor APOBEC3, which has antiretroviral activity, have higher viral loads after infection with murine leukaemia virus and corresponding higher levels of reverse viral transcripts and downstream interferon induction through the cGAS-STING pathway (cyclic GMP-AMP synthase-stimulator of interferon genes pathway)<sup>120</sup>. Similarly, SAMHD1 activity in vivo controls lentivirus load and limits virus-induced production of interferons in myeloid cells<sup>121</sup>. In addition, SAMHD1 deficiency leads to increased expression of costimulatory molecules and T cell activation on lentiviral infection, which suggests that the constitutive reduction of PRR activation by SAMHD1 limits not only the expression of innate immune cytokines but also downstream adaptive immune responses  $\frac{121}{2}$ . A third example is provided by the observation that expression of *Drosophila* Dicer in mammalian cells leads to decreased induction of IFNB by double-stranded RNA, most likely owing to the digestion of immunostimulatory RNA into shorter 20-25-bp RNA species that activate PRRs only

inefficiently<sup>122</sup>. Finally, constitutive innate immune mechanisms can also reduce PRR activity by lowering the concentration of PAMPs that have immunostimulatory activity. For example, lactoferrin binds CpG DNAs and inhibits their ability to activate TLR9 (ref.<sup>123</sup>).

#### Inhibition of PRR signalling

In addition to reducing the levels of PAMPs, some constitutive mechanisms have been reported to target PRR activity at the signalling level (Fig. 2a). For example, autophagy negatively regulates signalling by the RIG-I-MAVS pathway (retinoic acid-inducible gene I protein-mitochondrial antiviral signalling protein pathway) and by the cGAS-STING pathway; in the former case by limiting reactive oxygen species-mediated amplification of signalling and by LC3-dependent MAVS inactivation<sup>124,125</sup>, and in the latter case through degradation of STING<sup>126</sup>. In line with this, defective autophagy as a result of ATG16L deficiency predisposes to STING-dependent intestinal pathology in mice<sup>127</sup>, and ATG5 deficiency selectively in neutrophils exacerbates *M. tuberculosis* immunopathology without affecting bacterial load<sup>97</sup>. As a second example, lactate, which is produced during aerobic glycolysis and has virus-restricting activity<sup>73,74</sup>, also directly inhibits MAVS activity; thus lactate both reduces levels of viral PAMPs and has a negative regulatory function to inhibit PAMP-driven signalling and interferon expression<sup>128</sup>. Third, an engineered amphipathichelical antimicrobial peptide was found to block TLR4 signalling through the TRIF pathway<sup>129</sup>. This occurs by the inhibition of TLR4 endocytosis, which is an essential step for the engagement of TRIF from endosomal compartments.

Collectively, the current literature suggests that constitutive immune mechanisms reduce PRR activation through a range of mechanisms and, therefore, that these constitutive mechanisms impose a threshold and negative regulatory activity on the amplificative innate and adaptive immune responses (Fig. <u>2b</u>). We propose that rapid, molecularly specific and non-amplificative responses to challenges provided by constitutive immune mechanisms are beneficial for achieving optimal host defence with minimal immunopathology.

#### Constitutive immunity beyond infection

Our main focus here has been on infections. However, constitutive immune mechanisms are also involved in the elimination of sterile danger. For example, DNA damage in the nucleus and the accumulation of DNA in extranuclear compartments are eliminated by the DNA damage response and specific DNases<sup>130</sup>, respectively; the accumulation of misfolded proteins leads to the formation of aggresomes, which are cleared by selective autophagy $\frac{131,132}{132}$ ; excessive accumulation of reactive oxygen species leads to death of the oxygen-stressed cells<sup>133</sup>; and free cholesterol is converted into an ester derivative by lecithin–cholesterol acyltransferase, thus enabling transport to the liver by high-density lipoprotein and eventual degradation<sup>134</sup>. Defects in these constitutive and latent danger-eliminating mechanisms lead to the accumulation of danger-associated molecular patterns and activation of PRR-based immunity. For example, in cells with defects in either the DNA damage response or extranuclear DNases, the accumulation of DNA induces type I interferon production through the cGAS–STING pathway<sup>135,136,137,138</sup>. Similarly, defective elimination of protein aggregates or cholesterol leads to the induction of IL-1ß production through activation of the NLRP3 inflammasome<sup>139,140</sup>. Common to all of the examples given above is that the accumulated abnormal endogenous molecules are detected and eliminated through molecularly specific mechanisms independently of PRRs. This elimination limits PRR activation and hence inflammatory reactions. Therefore, in addition to eliminating microorganisms and PAMPs,

constitutive immune mechanisms also eliminate sterile danger signals in a damage-limiting manner that prevents the activation of excessive inflammation.

#### Constitutive immunity in human health

We propose that constitutive immune mechanisms enable cells and organisms to fight infections and eliminate endogenous abnormalities in a non-inflammatory manner. Therefore, an important benefit of these mechanisms may be to increase the threshold for development of clinically overt signs of disease on exposure to infections or endogenous danger. Studies of the associations between single-nucleotide polymorphisms and infections have shown that restriction factors, antimicrobial peptides and autophagy have important roles in antimicrobial defence<sup>141,142,143,144</sup>. Constitutive immune mechanisms may be particularly active in the protection of tissues that are frequently exposed to pathogens, such as epithelial cells in the airways and the gut, or tissues that are particularly vulnerable to immunopathology, such as the brain. In favour of this idea, RNA lariat debranching enzyme 1 (DBR1) and small nucleolar RNA, H/ACA box 31 (SNORA31) were recently shown to have non-redundant, interferon-independent roles in the prevention of viral brainstem encephalitis and herpes simplex encephalitis, respectively<sup>11,12</sup>. The mechanisms through which they exert their antiviral activity remain to be determined. Reports have shown that autophagy is an antiviral mechanism in the brain in mice  $\frac{36,89,145}{10}$ . In addition, some cell populations, including stem cells, seem to use constitutive immune mechanisms to eliminate danger without losing key functions, such as self-renewal and differentiation capacity, that are known to be impaired by PRR-based immunity<sup>146,147</sup>.

An important question related to human immunology is how individuals with a loss-offunction mutation in a constitutive immune mechanism may present clinically. Deficiency of a mechanism that is expressed in specific organs or cell types might lead to a higher frequency of clinical infections by a subset of microorganisms that are normally controlled by the defective mechanism. This seems to be the case for defects in DBR1, which confer susceptibility to disease caused by infections with herpes simplex virus type 1, influenza virus or norovirus in the brainstem<sup>11</sup>. The impact of deficiencies in constitutive immune mechanisms might not be limited to acute infections and could also include chronic and latent infections. In support of a link between such defects and increased inflammation, patients with inborn defects in DNA repair, elimination of extranuclear DNA or degradation of misfolded proteins develop autoinflammatory diseases, including Aicardi-Goutières syndrome and proteasome-associated autoinflammatory syndromes, which are characterized by type I interferon-dependent autoinflammation and are termed 'interferonopathies'<u>137,148,149,150</u>. Therefore, a loss of function in constitutive immune mechanisms can lead to selective susceptibility to specific infections or to infections in specific organs. Likewise, such deficiency might lead to the accumulation of PAMPs, microorganism-associated molecular patterns, danger-associated molecular patterns and/or homeostasis-altering molecular processes and associated pathological inflammation (Box 1).

#### Outlook

In this article, we have described the role and mode of action of a large panel of constitutive mechanisms used by the immune system to exert immediate control of infections and endogenous dangers independently of the inducible mechanisms that are activated through PRRs and antigen-specific receptors. Although many such constitutive responses have been known for years, greater understanding of the mechanisms involved and renewed interest in

fields such as restriction factor biology and immunometabolism are spurring further work in the area. With the identification of constitutive mechanisms that have non-redundant roles in host defence, we now know that these immune mechanisms are not just redundant, non-specific players in immunology<sup>11,12</sup>. This should stimulate interest in understanding the roles played by constitutive immune mechanisms in host defence in vivo, which might include the identification of new primary immune disorders. Improved knowledge of the host cell type and tissue specificities of constitutive immune mechanisms in relation to susceptibility to infections could greatly improve our understanding of human immunology. Such work will start to provide answers to the fundamental question of how the immune system determines the degree of threat caused by an infection and balances that with the appropriate strength of the immune reaction.

Finally, as we gain further insights into the various host responses that are activated during immunological challenge, it will be interesting to explore the idea that the immune system has a defensive layer of activities that have been selected to eliminate danger without engaging the PRR system (Box <u>3</u>). In this respect, it is interesting to note that in addition to the constitutive mechanisms described in this Review, there are various sensing systems that use transcriptional programmes to induce host defence independently of PRRs and with the ability to control inflammation. They include the NRF2–KEAP1, hypoxia-inducible factor 1 $\alpha$  and bone morphogenetic protein–SMAD pathways<sup>10,151,152,153</sup>. In addition, the constitutive host defence exerted by commensal bacteria through several mechanisms, including niche competition, warrants more attention. With more and more data emerging on the importance of constitutive mechanisms in immunology, there is a need to understand this phenomenon in more detail. Such work may advance our understanding of one of the most interesting questions in immunology, namely how to eliminate danger in a rapid, efficient and specific manner without causing excess damage to the host.

#### References

1. 1.

Medzhitov, R. Origin and physiological roles of inflammation. *Nature* **454**, 428–435 (2008).

CAS PubMed Google Scholar

2. 2.

van der Poll, T., van de Veerdonk, F. L., Scicluna, B. P. & Netea, M. G. The immunopathology of sepsis and potential therapeutic targets. *Nat. Rev. Immunol.* **17**, 407–420 (2017).

PubMed Google Scholar

3. 3.

Coban, C., Lee, M. S. J. & Ishii, K. J. Tissue-specific immunopathology during malaria infection. *Nat. Rev. Immunol.* **18**, 266–278 (2018).

CAS PubMed PubMed Central Google Scholar

4. 4.

Takeuchi, O. & Akira, S. Pattern recognition receptors and inflammation. *Cell* **140**, 805–820 (2010).

CAS PubMed Google Scholar

5. 5.

Iwasaki, A. & Medzhitov, R. Control of adaptive immunity by the innate immune system. *Nat. Immunol.* **16**, 343–353 (2015).

CAS PubMed PubMed Central Google Scholar

6. 6.

Flajnik, M. F. & Kasahara, M. Origin and evolution of the adaptive immune system: genetic events and selective pressures. *Nat. Rev. Genet.* **11**, 47–59 (2010).

CAS PubMed Google Scholar

7. 7.

Iversen, M. B. et al. An innate antiviral pathway acting before interferons at epithelial surfaces. *Nat. Immunol.* **17**, 150–158 (2016).

CAS PubMed Google Scholar

8. 8.

Yamane, D. et al. Basal expression of interferon regulatory factor 1 drives intrinsic hepatocyte resistance to multiple RNA viruses. *Nat. Microbiol.* **4**, 1096–1104 (2019).

CAS PubMed PubMed Central Google Scholar

9. 9.

Thurston, T. L., Ryzhakov, G., Bloor, S., von Muhlinen, N. & Randow, F. The TBK1 adaptor and autophagy receptor NDP52 restricts the proliferation of ubiquitin-coated bacteria. *Nat. Immunol.* **10**, 1215–1221 (2009).

CAS PubMed Google Scholar

10.10.

Eddowes, L. A. et al. Antiviral activity of bone morphogenetic proteins and activins. *Nat. Microbiol.* **4**, 339–351 (2019).

CAS PubMed Google Scholar

#### 11.11.

Zhang, S. Y. et al. Inborn errors of RNA lariat metabolism in humans with brainstem viral infection. *Cell* **172**, 952–965 (2018). Zhang et al. identify a genetic defect in a novel restriction mechanism that protects against viral brainstem infections.

CAS PubMed PubMed Central Google Scholar

12.12.

Lafaille, F. G. et al. Human SNORA31 variations impair cortical neuron-intrinsic immunity to HSV-1 and underlie herpes simplex encephalitis. *Nat. Med.* **25**, 1873–1884 (2019). This work identifies SNORA31 as an interferon-independent small antiviral nucleolar RNA conferring protection against herpes simplex encephalitis.

CAS PubMed PubMed Central Google Scholar

13.13.

Nish, S. & Medzhitov, R. Host defense pathways: role of redundancy and compensation in infectious disease phenotypes. *Immunity* **34**, 629–636 (2011).

CAS PubMed PubMed Central Google Scholar

14.14.

Ausubel, F. M. Are innate immune signaling pathways in plants and animals conserved? *Nat. Immunol.* **6**, 973–979 (2005).

CAS PubMed Google Scholar

15.15.

Matzinger, P. Tolerance, danger, and the extended family. *Annu. Rev. Immunol.* **12**, 991–1045 (1994).

CAS PubMed Google Scholar

16.16.

Liston, A. & Masters, S. L. Homeostasis-altering molecular processes as mechanisms of inflammasome activation. *Nat. Rev. Immunol.* **17**, 208–214 (2017).

CAS PubMed Google Scholar

17.17.

Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J. M. & Hoffmann, J. A. The dorsoventral regulatory gene cassette spätzle/Toll/cactus controls the potent antifungal response in Drosophila adults. *Cell* **86**, 973–983 (1996).

CAS PubMed Google Scholar

18.18.

Poltorak, A. et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* **282**, 2085–2088 (1999).

Google Scholar

19.19.

Crow, Y. J. & Manel, N. Aicardi-Goutieres syndrome and the type I interferonopathies. *Nat. Rev. Immunol.* **15**, 429–440 (2015).

CAS PubMed Google Scholar

20.20.

Dinarello, C. A., Simon, A. & van der Meer, J. W. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat. Rev. Drug Discov.* **11**, 633–652 (2012).

CAS PubMed PubMed Central Google Scholar

21.21.

Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S. & Medzhitov, R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* **118**, 229–241 (2004).

CAS PubMed Google Scholar

22.22.

Barton, E. S. et al. Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature* **447**, 326–329 (2007).

CAS PubMed Google Scholar

23.23.

Marie, I., Durbin, J. E. & Levy, D. E. Differential viral induction of distinct interferon-alpha genes by positive feedback through interferon regulatory factor-7. *EMBO J.* **17**, 6660–6669 (1998).

CAS PubMed PubMed Central Google Scholar

24.24.

Bauernfeind, F. G. et al. Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *J. Immunol.* **183**, 787–791 (2009).

CAS PubMed PubMed Central Google Scholar

25.25.

Yan, N., Regalado-Magdos, A. D., Stiggelbout, B., Lee-Kirsch, M. A. & Lieberman, J. The cytosolic exonuclease TREX1 inhibits the innate immune response to human immunodeficiency virus type 1. *Nat. Immunol.* **11**, 1005–1013 (2010).

CAS PubMed PubMed Central Google Scholar

26.26.

Luecke, S. et al. cGAS is activated by DNA in a length-dependent manner. *EMBO Rep.* **18**, 1707–1715 (2017).

CAS PubMed PubMed Central Google Scholar

27.27.

Gehrig, S. et al. Identification of modifications in microbial, native tRNA that suppress immunostimulatory activity. *J. Exp. Med.* **209**, 225–233 (2012).

CAS PubMed PubMed Central Google Scholar

28.28.

Rice, G. I. et al. Gain-of-function mutations in IFIH1 cause a spectrum of human disease phenotypes associated with upregulated type I interferon signaling. *Nat. Genet.* **46**, 503–509 (2014).

CAS PubMed PubMed Central Google Scholar

29.29.

Kagan, J. C., Magupalli, V. G. & Wu, H. SMOCs: supramolecular organizing centres that control innate immunity. *Nat. Rev. Immunol.* **14**, 821–826 (2014).

CAS PubMed PubMed Central Google Scholar

30.30.

Hamerman, J. A. et al. Negative regulation of TLR signaling in myeloid cellsimplications for autoimmune diseases. *Immunol. Rev.* **269**, 212–227 (2016). CAS PubMed PubMed Central Google Scholar

31.31.

Carey, C. M. et al. Recurrent loss-of-function mutations reveal costs to OAS1 antiviral activity in primates. *Cell Host Microbe* **25**, 336–343 (2019).

CAS PubMed PubMed Central Google Scholar

32.32.

Lim, J. K. et al. Genetic variation in OAS1 is a risk factor for initial infection with West Nile virus in man. *PLoS Pathog.* **5**, e1000321 (2009).

PubMed PubMed Central Google Scholar

33.33.

Li, H. et al. Identification of a Sjogren's syndrome susceptibility locus at OAS1 that influences isoform switching, protein expression, and responsiveness to type I interferons. *PLoS Genet.* **13**, e1006820 (2017).

PubMed PubMed Central Google Scholar

34.34.

Laguette, N. et al. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature* **474**, 654–657 (2011). **This work identifies SAMHD1 as an HIV-1 restriction factor that functions through a mechanism dependent on the phosphohydrolase activity of the enzyme**.

CAS PubMed PubMed Central Google Scholar

35.35.

Gariano, G. R. et al. The intracellular DNA sensor IFI16 gene acts as restriction factor for human cytomegalovirus replication. *PLoS Pathog.* **8**, e1002498 (2012).

CAS PubMed PubMed Central Google Scholar

36.36.

Orvedahl, A. et al. HSV-1 ICP34.5 confers neurovirulence by targeting the Beclin 1 autophagy protein. *Cell Host. Microbe* **1**, 23–35 (2007).

CAS PubMed Google Scholar

37.37.

Harris, R. S., Hultquist, J. F. & Evans, D. T. The restriction factors of human immunodeficiency virus. *J. Biol. Chem.* **287**, 40875–40883 (2012).

CAS PubMed PubMed Central Google Scholar

38.38.

Duggal, N. K. & Emerman, M. Evolutionary conflicts between viruses and restriction factors shape immunity. *Nat. Rev. Immunol.* **12**, 687–695 (2012).

CAS PubMed PubMed Central Google Scholar

39.39.

Bishop, K. N., Holmes, R. K., Sheehy, A. M. & Malim, M. H. APOBEC-mediated editing of viral RNA. *Science* **305**, 645 (2004). This study describes the identification of APOBEC-mediated RNA editing as a mechanism restricting HIV-1 replication.

CAS PubMed Google Scholar

40.40.

Neil, S. J., Zang, T. & Bieniasz, P. D. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* **451**, 425–430 (2008).

CAS PubMed Google Scholar

41.41.

Goldstone, D. C. et al. HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. *Nature* **480**, 379–382 (2011).

CAS PubMed Google Scholar

Glass, M. & Everett, R. D. Components of promyelocytic leukemia nuclear bodies (ND10) act cooperatively to repress herpesvirus infection. *J. Virol.* **87**, 2174–2185 (2013).

CAS PubMed PubMed Central Google Scholar

43.43.

Merkl, P. E. & Knipe, D. M. Role for a filamentous nuclear assembly of IFI16, DNA, and host factors in restriction of herpesviral infection. *mBio* **10**, e02621 (2019).

CAS PubMed PubMed Central Google Scholar

<sup>42.42.</sup> 

44.44.

Pichlmair, A. et al. IFIT1 is an antiviral protein that recognizes 5'-triphosphate RNA. *Nat. Immunol.* **12**, 624–630 (2011).

CAS PubMed Google Scholar

45.45.

Full, F. et al. Centrosomal protein TRIM43 restricts herpesvirus infection by regulating nuclear lamina integrity. *Nat. Microbiol.* **4**, 164–176 (2019).

CAS PubMed Google Scholar

46.46.

Schoggins, J. W. et al. Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. *Nature* **505**, 691–695 (2013).

PubMed PubMed Central Google Scholar

47.47.

Brien, J. D. et al. Interferon regulatory factor-1 (IRF-1) shapes both innate and CD8<sup>+</sup> T cell immune responses against West Nile virus infection. *PLoS Pathog.* **7**, e1002230 (2011).

CAS PubMed PubMed Central Google Scholar

48.48.

Zhou, R. & Rana, T. M. RNA-based mechanisms regulating host-virus interactions. *Immunol. Rev.* **253**, 97–111 (2013).

PubMed PubMed Central Google Scholar

49.49.

Hamilton, A. J. & Baulcombe, D. C. A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* **286**, 950–952 (1999).

CAS PubMed Google Scholar

50.50.

Mourrain, P. et al. Arabidopsis SGS2 and SGS3 genes are required for posttranscriptional gene silencing and natural virus resistance. *Cell* **101**, 533–542 (2000). **Mourrain et al. identify RNAi as an antiviral system in plants**.

CAS PubMed Google Scholar

51.51.

Lu, R. et al. Animal virus replication and RNAi-mediated antiviral silencing in *Caenorhabditis elegans. Nature* **436**, 1040–1043 (2005).

CAS PubMed PubMed Central Google Scholar

52.52.

Wang, X. H. et al. RNA interference directs innate immunity against viruses in adult Drosophila. *Science* **312**, 452–454 (2006).

CAS PubMed PubMed Central Google Scholar

53.53.

Galiana-Arnoux, D., Dostert, C., Schneemann, A., Hoffmann, J. A. & Imler, J. L. Essential function in vivo for Dicer-2 in host defense against RNA viruses in drosophila. *Nat. Immunol.* **7**, 590–597 (2006).

CAS PubMed Google Scholar

54.54.

Maillard, P. V., van der Veen, A. G., Poirier, E. Z. & Reis, E. S. C. Slicing and dicing viruses: antiviral RNA interference in mammals. *EMBO J.* **38**, e100941 (2019).

CAS PubMed PubMed Central Google Scholar

55.55.

Wang, Y. et al. Hepatitis C virus core protein is a potent inhibitor of RNA silencingbased antiviral response. *Gastroenterology* **130**, 883–892 (2006).

CAS PubMed Google Scholar

#### 56.56.

Fabozzi, G., Nabel, C. S., Dolan, M. A. & Sullivan, N. J. Ebolavirus proteins suppress the effects of small interfering RNA by direct interaction with the mammalian RNA interference pathway. *J. Virol.* **85**, 2512–2523 (2011).

CAS PubMed PubMed Central Google Scholar

57.57.

Yeaman, M. R. & Yount, N. Y. Mechanisms of antimicrobial peptide action and resistance. *Pharmacol. Rev.* **55**, 27–55 (2003).

CAS PubMed Google Scholar

58.58.

Wilson, C. L. et al. Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. *Science* **286**, 113–117 (1999).

CAS PubMed Google Scholar

59.59.

Chromek, M. et al. The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection. *Nat. Med.* **12**, 636–641 (2006).

CAS PubMed Google Scholar

60.60.

Ganz, T., Metcalf, J. A., Gallin, J. I., Boxer, L. A. & Lehrer, R. I. Microbicidal/cytotoxic proteins of neutrophils are deficient in two disorders: Chediak-Higashi syndrome and "specific" granule deficiency. *J. Clin. Invest.* **82**, 552–556 (1988).

CAS PubMed PubMed Central Google Scholar

61.61.

Kumar, P., Kizhakkedathu, J. N. & Straus, S. K. Antimicrobial peptides: diversity, mechanism of action and strategies to improve the activity and biocompatibility in vivo. *Biomolecules* **8**, 4 (2018).

PubMed Central Google Scholar

62.62.

Jenssen, H., Hamill, P. & Hancock, R. E. Peptide antimicrobial agents. *Clin. Microbiol. Rev.* **19**, 491–511 (2006).

CAS PubMed PubMed Central Google Scholar

63.63.

Valore, E. V. et al. Human beta-defensin-1: an antimicrobial peptide of urogenital tissues. *J. Clin. Invest.* **101**, 1633–1642 (1998).

CAS PubMed PubMed Central Google Scholar

64.64.

Nizet, V. et al. Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature* **414**, 454–457 (2001).

CAS PubMed Google Scholar

65.65.

Quinones-Mateu, M. E. et al. Human epithelial beta-defensins 2 and 3 inhibit HIV-1 replication. *AIDS* **17**, F39–F48 (2003).

CAS PubMed Google Scholar

66.66.

Ahmed, A., Siman-Tov, G., Hall, G., Bhalla, N. & Narayanan, A. Human antimicrobial peptides as therapeutics for viral infections. *Viruses* **11**, 704 (2019).

CAS PubMed Central Google Scholar

67.67.

Casals, C., Garcia-Fojeda, B. & Minutti, C. M. Soluble defense collagens: sweeping up immune threats. *Mol. Immunol.* **112**, 291–304 (2019).

CAS PubMed Google Scholar

68.68.

Meschi, J. et al. Surfactant protein D binds to human immunodeficiency virus (HIV) envelope protein gp120 and inhibits HIV replication. *J. Gen. Virol.* **86**, 3097–3107 (2005).

CAS PubMed Google Scholar

69.69.

Hartshorn, K. L. et al. Reduced influenza viral neutralizing activity of natural human trimers of surfactant protein D. *Respir. Res.* **8**, 9 (2007).

PubMed PubMed Central Google Scholar

70.70.

Reading, P. C. et al. Antiviral activity of the long chain pentraxin PTX3 against influenza viruses. *J. Immunol.* **180**, 3391–3398 (2008).

CAS PubMed Google Scholar

71.71.

LeVine, A. M., Whitsett, J. A., Hartshorn, K. L., Crouch, E. C. & Korfhagen, T. R. Surfactant protein D enhances clearance of influenza A virus from the lung in vivo. *J. Immunol.* **167**, 5868–5873 (2001).

#### CAS PubMed Google Scholar

72.72.

Jounblat, R. et al. Binding and agglutination of *Streptococcus pneumoniae* by human surfactant protein D (SP-D) vary between strains, but SP-D fails to enhance killing by neutrophils. *Infect. Immun.* **72**, 709–716 (2004).

CAS PubMed PubMed Central Google Scholar

73.73.

Isaacs, C. E. & Xu, W. Theaflavin-3,3'-digallate and lactic acid combinations reduce herpes simplex virus infectivity. *Antimicrob. Agents. Chemother.* **57**, 3806–3814 (2013).

CAS PubMed PubMed Central Google Scholar

74.74.

Tyssen, D. et al. Anti-HIV-1 activity of lactic acid in human cervicovaginal fluid. *mSphere* **3**, e00055 (2018).

CAS PubMed PubMed Central Google Scholar

75.75.

Sanchez, E. L. & Lagunoff, M. Viral activation of cellular metabolism. *Virology* **479-480**, 609–618 (2015).

CAS PubMed Google Scholar

76.76.

Munger, J., Bajad, S. U., Coller, H. A., Shenk, T. & Rabinowitz, J. D. Dynamics of the cellular metabolome during human cytomegalovirus infection. *PLoS Pathog.* **2**, e132 (2006).

PubMed PubMed Central Google Scholar

77.77.

Libran-Perez, M., Pereiro, P., Figueras, A. & Novoa, B. Antiviral activity of palmitic acid via autophagic flux inhibition in zebrafish (*Danio rerio*). *Fish Shellfish Immunol.* **95**, 595–605 (2019).

CAS PubMed Google Scholar

78.78.

Kachroo, A. et al. An oleic acid-mediated pathway induces constitutive defense signaling and enhanced resistance to multiple pathogens in soybean. *Mol. Plant Microbe Interact.* **21**, 564–575 (2008).

CAS PubMed Google Scholar

79.79.

Nevo, Y. & Nelson, N. The NRAMP family of metal-ion transporters. *Biochim. Biophys. Acta* **1763**, 609–620 (2006).

CAS PubMed Google Scholar

80.80.

Vidal, S. M., Malo, D., Vogan, K., Skamene, E. & Gros, P. Natural resistance to infection with intracellular parasites: isolation of a candidate for Bcg. *Cell* **73**, 469–485 (1993).

CAS PubMed Google Scholar

81.81.

Plant, J. E., Blackwell, J. M., O'Brien, A. D., Bradley, D. J. & Glynn, A. A. Are the Lsh and Ity disease resistance genes at one locus on mouse chromosome 1? *Nature* **297**, 510–511 (1982).

CAS PubMed Google Scholar

82.82.

Supek, F., Supekova, L., Nelson, H. & Nelson, N. A yeast manganese transporter related to the macrophage protein involved in conferring resistance to mycobacteria. *Proc. Natl Acad. Sci. USA* **93**, 5105–5110 (1996).

CAS PubMed Google Scholar

83.83.

Mayeur, S., Spahis, S., Pouliot, Y. & Levy, E. Lactoferrin, a pleiotropic protein in health and disease. *Antioxid. Redox Signal.* **24**, 813–836 (2016).

CAS PubMed Google Scholar

84.84.

Velusamy, S. K., Markowitz, K., Fine, D. H. & Velliyagounder, K. Human lactoferrin protects against *Streptococcus mutans*-induced caries in mice. *Oral Dis.* **22**, 148–154 (2016).

#### CAS PubMed Google Scholar

85.85.

Levine, B., Mizushima, N. & Virgin, H. W. Autophagy in immunity and inflammation. *Nature* **469**, 323–335 (2011).

CAS PubMed PubMed Central Google Scholar

86.86.

Lim, J. J., Grinstein, S. & Roth, Z. Diversity and versatility of phagocytosis: roles in innate immunity, tissue remodeling, and homeostasis. *Front. Cell. Infect. Microbiol.* **7**, 191 (2017).

PubMed PubMed Central Google Scholar

87.87.

Thurston, T. L. M., Wandel, M. P., von Muhlinen, N., Foeglein, A. & Randow, F. Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. *Nature* **482**, 414–418 (2012).

CAS PubMed PubMed Central Google Scholar

88.88.

Gros, P., Milder, F. J. & Janssen, B. J. Complement driven by conformational changes. *Nat. Rev. Immunol.* **8**, 48–58 (2008).

CAS PubMed Google Scholar

89.89.

Orvedahl, A. et al. Autophagy protects against Sindbis virus infection of the central nervous system. *Cell Host Microbe* **7**, 115–127 (2010). This study identifies an essential role for autophagy in antiviral defence in vitro and in vivo in mice.

CAS PubMed PubMed Central Google Scholar

90.90.

Sparrer, K. M. J. et al. TRIM23 mediates virus-induced autophagy via activation of TBK1. *Nat. Microbiol.* **2**, 1543–1557 (2017).

CAS PubMed PubMed Central Google Scholar

91.91.

Franco, L. H. et al. The ubiquitin ligase Smurf1 functions in selective autophagy of *Mycobacterium tuberculosis* and anti-tuberculous host defense. *Cell Host Microbe* **21**, 59–72 (2017).

CAS PubMed Google Scholar

92.92.

Huett, A. et al. The LRR and RING domain protein LRSAM1 is an E3 ligase crucial for ubiquitin-dependent autophagy of intracellular *Salmonella* Typhimurium. *Cell Host Microbe* **12**, 778–790 (2012).

CAS PubMed PubMed Central Google Scholar

93.93.

Wild, P. et al. Phosphorylation of the autophagy receptor optineurin restricts Salmonella growth. *Science* **333**, 228–233 (2011).

CAS PubMed PubMed Central Google Scholar

94.94.

Ravenhill, B. J. et al. The cargo receptor NDP52 initiates selective autophagy by recruiting the ULK complex to cytosol-invading bacteria. *Mol. Cell* **74**, 320–329 (2019).

CAS PubMed PubMed Central Google Scholar

95.95.

Gutierrez, M. G. et al. Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell* **119**, 753–766 (2004). This work provides the first description of autophagy as an antibacterial mechanism.

CAS PubMed Google Scholar

96.96.

Castillo, E. F. et al. Autophagy protects against active tuberculosis by suppressing bacterial burden and inflammation. *Proc. Natl Acad. Sci. USA* **109**, E3168–E3176 (2012).

CAS PubMed Google Scholar

97.97.

Kimmey, J. M. et al. Unique role for ATG5 in neutrophil-mediated immunopathology during *M. tuberculosis* infection. *Nature* **528**, 565–569 (2015).

CAS PubMed PubMed Central Google Scholar

98.98.

Ricklin, D., Reis, E. S. & Lambris, J. D. Complement in disease: a defence system turning offensive. *Nat. Rev. Nephrol.* **12**, 383–401 (2016).

CAS PubMed PubMed Central Google Scholar

99.99.

Shi, L. et al. Mannose-binding lectin-deficient mice are susceptible to infection with *Staphylococcus aureus*. J. Exp. Med. **199**, 1379–1390 (2004).

CAS PubMed PubMed Central Google Scholar

100. 100.

Whitnack, E. & Beachey, E. H. Inhibition of complement-mediated opsonization and phagocytosis of *Streptococcus pyogenes* by D fragments of fibrinogen and fibrin bound to cell surface M protein. *J. Exp. Med.* **162**, 1983–1997 (1985).

CAS PubMed Google Scholar

101. 101.

Heckmann, B. L., Boada-Romero, E., Cunha, L. D., Magne, J. & Green, D. R. LC3-associated phagocytosis and inflammation. *J. Mol. Biol.* **429**, 3561–3576 (2017).

CAS PubMed PubMed Central Google Scholar

102. 102.

Martinez, J. et al. Noncanonical autophagy inhibits the autoinflammatory, lupus-like response to dying cells. *Nature* **533**, 115–119 (2016).

CAS PubMed PubMed Central Google Scholar

103. 103.

Martinez, J. et al. Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins. *Nat. Cell. Biol.* **17**, 893–906 (2015).

CAS PubMed PubMed Central Google Scholar

104. 104.

Wang, Y. & Le, W. D. Autophagy and ubiquitin-proteasome system. *Adv. Exp. Med. Biol.* **1206**, 527–550 (2019).

#### CAS PubMed Google Scholar

105. 105.

Hauler, F., Mallery, D. L., McEwan, W. A., Bidgood, S. R. & James, L. C. AAA ATPase p97/VCP is essential for TRIM21-mediated virus neutralization. *Proc. Natl Acad. Sci. USA* **109**, 19733–19738 (2012). These authors identify an important role for the ubiquitin–proteasome pathway in cytosolic neutralization of viral capsids.

CAS PubMed Google Scholar

106. 106.

Tam, J. C., Bidgood, S. R., McEwan, W. A. & James, L. C. Intracellular sensing of complement C3 activates cell autonomous immunity. *Science* **345**, 1256070 (2014).

PubMed PubMed Central Google Scholar

107. 107.

Bottermann, M. et al. Complement C4 prevents viral infection through capsid inactivation. *Cell Host Microbe* **25**, 617–629 e617 (2019).

CAS PubMed PubMed Central Google Scholar

108. 108.

Camborde, L. et al. The ubiquitin-proteasome system regulates the accumulation of Turnip yellow mosaic virus RNA-dependent RNA polymerase during viral infection. *Plant Cell* **22**, 3142–3152 (2010).

CAS PubMed PubMed Central Google Scholar

109. 109.

Ruckdeschel, K. et al. The proteasome pathway destabilizes Yersinia outer protein E and represses its antihost cell activities. *J. Immunol.* **176**, 6093–6102 (2006).

CAS PubMed Google Scholar

110. 110.

Sahana, N. et al. Inhibition of the host proteasome facilitates papaya ringspot virus accumulation and proteosomal catalytic activity is modulated by viral factor HcPro. *PLoS ONE* **7**, e52546 (2012).

CAS PubMed PubMed Central Google Scholar

111. 111.

Xu, Y. et al. Rice stripe tenuivirus nonstructural protein 3 hijacks the 26S proteasome of the small brown planthopper via direct interaction with regulatory particle non-ATPase subunit 3. *J. Virol.* **89**, 4296–4310 (2015).

CAS PubMed PubMed Central Google Scholar

112. 112.

Dudnik, A., Bigler, L. & Dudler, R. Production of proteasome inhibitor syringolin A by the endophyte Rhizobium sp. strain AP16. *Appl. Environ. Microbiol.* **80**, 3741–3748 (2014).

PubMed PubMed Central Google Scholar

113. 113.

Groll, M. et al. A plant pathogen virulence factor inhibits the eukaryotic proteasome by a novel mechanism. *Nature* **452**, 755–758 (2008).

CAS PubMed Google Scholar

114. 114.

Zimmermann, C. et al. The abundant tegument protein pUL25 of human cytomegalovirus prevents proteasomal degradation of pUL26 and supports its suppression of ISGylation. *J. Virol.* **92**, e01180–e01218 (2018).

CAS PubMed PubMed Central Google Scholar

115. 115.

Chakrabarti, A., Jha, B. K. & Silverman, R. H. New insights into the role of RNase L in innate immunity. *J. Interferon Cytokine Res.* **31**, 49–57 (2011).

CAS PubMed PubMed Central Google Scholar

116. 116.

Banerjee, S. et al. OAS-RNase L innate immune pathway mediates the cytotoxicity of a DNA-demethylating drug. *Proc. Natl Acad. Sci. USA* **116**, 5071–5076 (2019).

CAS PubMed Google Scholar

117. 117.

Birdwell, L. D. et al. Activation of RNase L by murine coronavirus in myeloid cells is dependent on basal Oas gene expression and independent of virus-induced interferon. *J. Virol.* **90**, 3160–3172 (2016).

PubMed PubMed Central Google Scholar

#### 118. 118.

Stetson, D. B., Ko, J. S., Heidmann, T. & Medzhitov, R. Trex1 prevents cell-intrinsic initiation of autoimmunity. *Cell* **134**, 587–598 (2008).

CAS PubMed PubMed Central Google Scholar

119. 119.

Mogensen, T. H. Pathogen recognition and inflammatory signaling in innate immune defenses. *Clin. Microbiol. Rev.* **22**, 240–273 (2009).

CAS PubMed PubMed Central Google Scholar

120. 120.

Stavrou, S., Blouch, K., Kotla, S., Bass, A. & Ross, S. R. Nucleic acid recognition orchestrates the anti-viral response to retroviruses. *Cell Host Microbe* **17**, 478–488 (2015). Stavrou et al. show that lack of the restriction factor APOBEC3 leads to higher load of retroviral nucleic acids, and increased STING-dependent IFNβ expression.

CAS PubMed PubMed Central Google Scholar

121. 121.

Maelfait, J., Bridgeman, A., Benlahrech, A., Cursi, C. & Rehwinkel, J. Restriction by SAMHD1 limits cGAS/STING-dependent innate and adaptive immune responses to HIV-1. *Cell Rep.* **16**, 1492–1501 (2016). **This work shows that SAMHD1 limits lentivirus-induced type I interferon production and T cell cytotoxicity, thus providing direct evidence for constitutive immune responses limiting inducible immune activities.** 

CAS PubMed PubMed Central Google Scholar

122. 122.

Marques, J. T. et al. A structural basis for discriminating between self and nonself double-stranded RNAs in mammalian cells. *Nat. Biotechnol.* **24**, 559–565 (2006).

CAS PubMed Google Scholar

123. 123.

Britigan, B. E., Lewis, T. S., Waldschmidt, M., McCormick, M. L. & Krieg, A. M. Lactoferrin binds CpG-containing oligonucleotides and inhibits their immunostimulatory effects on human B cells. *J. Immunol.* **167**, 2921–2928 (2001).

CAS PubMed Google Scholar

124. 124.

Cheng, J. et al. Autophagy regulates MAVS signaling activation in a phosphorylationdependent manner in microglia. *Cell Death Differ.* **24**, 276–287 (2017).

CAS PubMed Google Scholar

125. 125.

Tal, M. C. et al. Absence of autophagy results in reactive oxygen species-dependent amplification of RLR signaling. *Proc. Natl Acad. Sci. USA* **106**, 2770–2775 (2009).

CAS PubMed Google Scholar

126. 126.

Prabakaran, T. et al. Attenuation of cGAS-STING signaling is mediated by a p62/SQSTM1-dependent autophagy pathway activated by TBK1. *EMBO J.* **37**, e97858 (2018). Cheng et al. (2017), Tal et al. (2009) and Prabakaran et al. show that autophagy directly inhibits signalling by the RIG-I-like receptor–MAVS and cGAS–STING pathways.

PubMed PubMed Central Google Scholar

127. 127.

Aden, K. et al. ATG16L1 orchestrates interleukin-22 signaling in the intestinal epithelium via cGAS-STING. *J. Exp. Med.* **215**, 2868–2886 (2018).

CAS PubMed PubMed Central Google Scholar

128. 128.

Zhang, W. et al. Lactate is a natural suppressor of RLR signaling by targeting MAVS. *Cell* **178**, 176–189.e15 (2019). This report shows that lactate directly inhibits RIG-I-like receptor–MAVS signalling.

CAS PubMed PubMed Central Google Scholar

129. 129.

Shim, D. W. et al. Anti-inflammatory action of an antimicrobial model peptide that suppresses the TRIF-dependent signaling pathway via inhibition of toll-like receptor 4 endocytosis in lipopolysaccharide-stimulated macrophages. *PLoS ONE* **10**, e0126871 (2015).

PubMed PubMed Central Google Scholar

130. 130.

Haber, J. E. Deciphering the DNA damage response. Cell 162, 1183-1185 (2015).

CAS PubMed Google Scholar

131. 131.

Johnston, J. A., Ward, C. L. & Kopito, R. R. Aggresomes: a cellular response to misfolded proteins. *J. Cell. Biol.* **143**, 1883–1898 (1998).

CAS PubMed PubMed Central Google Scholar

132. 132.

Fortun, J., Dunn, W. A. Jr, Joy, S., Li, J. & Notterpek, L. Emerging role for autophagy in the removal of aggresomes in Schwann cells. *J. Neurosci.* 23, 10672–10680 (2003).

CAS PubMed PubMed Central Google Scholar

133. 133.

Holze, C. et al. Oxeiptosis, a ROS-induced caspase-independent apoptosis-like cell-death pathway. *Nat. Immunol.* **19**, 130–140 (2018).

CAS PubMed Google Scholar

134. 134.

Yu, X. H., Zhang, D. W., Zheng, X. L. & Tang, C. K. Cholesterol transport system: an integrated cholesterol transport model involved in atherosclerosis. *Prog. Lipid Res.* **73**, 65–91 (2019).

CAS PubMed Google Scholar

135. 135.

Mackenzie, K. J. et al. cGAS surveillance of micronuclei links genome instability to innate immunity. *Nature* **548**, 461–465 (2017).

CAS PubMed PubMed Central Google Scholar

136. 136.

Harding, S. M. et al. Mitotic progression following DNA damage enables pattern recognition within micronuclei. *Nature* **548**, 466–470 (2017).

CAS PubMed PubMed Central Google Scholar

137. 137.

Crow, Y. J. et al. Mutations in the gene encoding the 3'-5' DNA exonuclease TREX1 cause Aicardi-Goutieres syndrome at the AGS1 locus. *Nat. Genet.* **38**, 917–920 (2006). Loss-of-function mutations in the gene encoding the DNA exonuclease TREX1 lead to constitutive type I interferon signalling.

CAS PubMed Google Scholar

138. 138.

Rodero, M. P. et al. Type I interferon-mediated autoinflammation due to DNase II deficiency. *Nat. Commun.* **8**, 2176 (2017).

PubMed PubMed Central Google Scholar

139. 139.

Halle, A. et al. The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. *Nat. Immunol.* **9**, 857–865 (2008).

CAS PubMed PubMed Central Google Scholar

140. 140.

Duewell, P. et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* **464**, 1357–1361 (2010).

CAS PubMed PubMed Central Google Scholar

141. 141.

Laplana, M., Caruz, A., Pineda, J. A., Puig, T. & Fibla, J. Association of BST-2 gene variants with HIV disease progression underscores the role of BST-2 in HIV type 1 infection. *J. Infect. Dis.* **207**, 411–419 (2013).

CAS PubMed Google Scholar

142. 142.

Everitt, A. R. et al. IFITM3 restricts the morbidity and mortality associated with influenza. *Nature* **484**, 519–523 (2012).

CAS PubMed PubMed Central Google Scholar

143. 143.

Tesse, R. et al. Association of beta-defensin-1 gene polymorphisms with *Pseudomonas aeruginosa* airway colonization in cystic fibrosis. *Genes Immun.* **9**, 57–60 (2008).

CAS PubMed Google Scholar

144. 144.

Shao, Y. et al. Association between genetic polymorphisms in the autophagy-related 5 gene promoter and the risk of sepsis. *Sci. Rep.* **7**, 9399 (2017).

PubMed PubMed Central Google Scholar

145. 145.

Yordy, B., Iijima, N., Huttner, A., Leib, D. & Iwasaki, A. A neuron-specific role for autophagy in antiviral defense against herpes simplex virus. *Cell Host Microbe* **12**, 334–345 (2012).

CAS PubMed PubMed Central Google Scholar

146. 146.

Wu, X. et al. Intrinsic immunity shapes viral resistance of stem cells. *Cell* **172**, 423–438 e425 (2018).

CAS PubMed Google Scholar

147. 147.

Eggenberger, J., Blanco-Melo, D., Panis, M., Brennand, K. J. & Tenoever, B. R. Type I interferon response impairs differentiation potential of pluripotent stem cells. *Proc. Natl Acad. Sci. USA* **116**, 1384–1393 (2019).

CAS PubMed Google Scholar

148. 148.

Liu, Y. et al. Mutations in proteasome subunit beta type 8 cause chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature with evidence of genetic and phenotypic heterogeneity. *Arthritis Rheum.* **64**, 895–907 (2012).

CAS PubMed PubMed Central Google Scholar

149. 149.

Brehm, A. et al. Additive loss-of-function proteasome subunit mutations in CANDLE/PRAAS patients promote type I IFN production. *J. Clin. Invest.* **125**, 4196–4211 (2015). These authors report that patients with mutations in genes encoding proteasome subunits develop disease with a type I interferon signature.

PubMed PubMed Central Google Scholar

150. 150.

Massaad, M. J. et al. Deficiency of base excision repair enzyme NEIL3 drives increased predisposition to autoimmunity. *J. Clin. Invest.* **126**, 4219–4236 (2016).

PubMed PubMed Central Google Scholar

151. 151.

Khor, T. O. et al. Nrf2-deficient mice have an increased susceptibility to dextran sulfate sodium-induced colitis. *Cancer Res.* **66**, 11580–11584 (2006).

CAS PubMed Google Scholar

152. 152.

Ivanciuc, T., Sbrana, E., Casola, A. & Garofalo, R. P. Protective role of nuclear factor erythroid 2-related factor 2 against respiratory syncytial virus and human metapneumovirus infections. *Front. Immunol.* **9**, 854 (2018).

PubMed PubMed Central Google Scholar

153. 153.

Peyssonnaux, C. et al. HIF-1alpha expression regulates the bactericidal capacity of phagocytes. *J. Clin. Invest.* **115**, 1806–1815 (2005).

CAS PubMed PubMed Central Google Scholar

154. 154.

Blondeau, C. et al. Tetherin restricts herpes simplex virus 1 and is antagonized by glycoprotein M. *J. Virol.* **87**, 13124–13133 (2013).

CAS PubMed PubMed Central Google Scholar

155. 155.

Smith, S. E. et al. Interferon-induced transmembrane protein 1 restricts replication of viruses that enter cells via the plasma membrane. *J. Virol.* **93**, e02003 (2019).

CAS PubMed PubMed Central Google Scholar

156. 156.

Bernhardt, A. et al. Inflammatory cell infiltration and resolution of kidney inflammation is orchestrated by the cold-shock protein Y-box binding protein-1. *Kidney Int.* **92**, 1157–1177 (2017).

CAS PubMed Google Scholar

157. 157.

Hollenbaugh, J. A. et al. Host factor SAMHD1 restricts DNA viruses in non-dividing myeloid cells. *PLoS Pathog.* **9**, e1003481 (2013).

CAS PubMed PubMed Central Google Scholar

158. 158.

Nakaya, Y., Stavrou, S., Blouch, K., Tattersall, P. & Ross, S. R. In vivo examination of mouse APOBEC3- and human APOBEC3A- and APOBEC3G-mediated restriction of parvovirus and herpesvirus infection in mouse models. *J. Virol.* **90**, 8005–8012 (2016).

CAS PubMed PubMed Central Google Scholar

159. 159.

Girardi, E. et al. Cross-species comparative analysis of Dicer proteins during Sindbis virus infection. *Sci. Rep.* **5**, 10693 (2015).

PubMed PubMed Central Google Scholar

160. 160.

Dombrowski, Y. et al. Cytosolic DNA triggers inflammasome activation in keratinocytes in psoriatic lesions. *Sci. Transl. Med.* **3**, 82ra38 (2011).

PubMed PubMed Central Google Scholar

161. 161.

Stamme, C., Muller, M., Hamann, L., Gutsmann, T. & Seydel, U. Surfactant protein a inhibits lipopolysaccharide-induced immune cell activation by preventing the interaction of lipopolysaccharide with lipopolysaccharide-binding protein. *Am. J. Respir. Cell Mol. Biol.* **27**, 353–360 (2002).

CAS PubMed Google Scholar

162. 162.

Daniels, B. P. et al. The nucleotide sensor ZBP1 and kinase RIPK3 induce the enzyme IRG1 to promote an antiviral metabolic state in neurons. *Immunity* **50**, 64–76 e64 (2019).

CAS PubMed PubMed Central Google Scholar

163. 163.

Nair, S. et al. Irg1 expression in myeloid cells prevents immunopathology during *M. tuberculosis* infection. *J. Exp. Med.* **215**, 1035–1045 (2018).

#### CAS PubMed PubMed Central Google Scholar

164. 164.

Jessop, F., Hamilton, R. F., Rhoderick, J. F., Shaw, P. K. & Holian, A. Autophagy deficiency in macrophages enhances NLRP3 inflammasome activity and chronic lung disease following silica exposure. *Toxicol. Appl. Pharmacol.* **309**, 101–110 (2016).

CAS PubMed PubMed Central Google Scholar

165. 165.

Meissner, F. et al. Inflammasome activation in NADPH oxidase defective mononuclear phagocytes from patients with chronic granulomatous disease. *Blood* **116**, 1570–1573 (2010).

CAS PubMed PubMed Central Google Scholar

166. 166.

Segal, B. H. et al. NADPH oxidase limits innate immune responses in the lungs in mice. *PLoS ONE* **5**, e9631 (2010).

PubMed PubMed Central Google Scholar

167. 167.

Gluschko, A. et al. The beta2 integrin Mac-1 induces protective LC3-associated phagocytosis of listeria monocytogenes. *Cell Host Microbe* **23**, 324–337 e325 (2018).

CAS PubMed Google Scholar

168. 168.

Gong, L. et al. The *Burkholderia pseudomallei* type III secretion system and BopA are required for evasion of LC3-associated phagocytosis. *PLoS ONE* **6**, e17852 (2011).

CAS PubMed PubMed Central Google Scholar

169. 169.

Masters, S. L., Simon, A., Aksentijevich, I. & Kastner, D. L. Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease. *Ann. Rev. Immunol.* **27**, 621–668 (2009).

CAS Google Scholar

170. 170.

Uggenti, C., Lepelley, A. & Crow, Y. J. Self-awareness: nucleic acid-driven inflammation and the type I interferonopathies. *Annu. Rev. Immunol.* **37**, 247–267 (2019).

CAS PubMed Google Scholar

171. 171.

Jesus, A. A. & Goldbach-Mansky, R. IL-1 blockade in autoinflammatory syndromes. *Annu. Rev. Med.* **65**, 223–244 (2014).

CAS PubMed PubMed Central Google Scholar

172. 172.

Schwartz, D. M. et al. JAK inhibition as a therapeutic strategy for immune and inflammatory diseases. *Nat. Rev. Drug. Discov.* **17**, 78 (2017).

PubMed PubMed Central Google Scholar

173. 173.

Kim, H., Sanchez, G. A. & Goldbach-Mansky, R. Insights from Mendelian interferonopathies: comparison of CANDLE, SAVI with AGS, monogenic lupus. *J. Mol. Med.* **94**, 1111–1127 (2016).

CAS PubMed PubMed Central Google Scholar

174. 174.

Sanjuan, M. A. et al. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature* **450**, 1253–1257 (2007).

CAS PubMed Google Scholar

175. 175.

Doyle, S. E. et al. Toll-like receptors induce a phagocytic gene program through p38. *J. Exp. Med.* **199**, 81–90 (2004).

CAS PubMed PubMed Central Google Scholar

176. 176.

Henneke, P. et al. Cellular activation, phagocytosis, and bactericidal activity against group B streptococcus involve parallel myeloid differentiation factor 88-dependent and independent signaling pathways. *J. Immunol.* **169**, 3970–3977 (2002).

CAS PubMed Google Scholar

#### 177. 177.

Hawley, K. L. et al. CD14 cooperates with complement receptor 3 to mediate MyD88independent phagocytosis of *Borrelia burgdorferi*. *Proc. Natl Acad. Sci. USA* **109**, 1228–1232 (2012).

CAS PubMed Google Scholar

178. 178.

Peng, G., Lei, K. J., Jin, W., Greenwell-Wild, T. & Wahl, S. M. Induction of APOBEC3 family proteins, a defensive maneuver underlying interferon-induced anti-HIV-1 activity. *J. Exp. Med.* **203**, 41–46 (2006).

CAS PubMed PubMed Central Google Scholar

179. 179.

Walmsley, S. R. et al. Prolyl hydroxylase 3 (PHD3) is essential for hypoxic regulation of neutrophilic inflammation in humans and mice. *J. Clin. Invest.* **121**, 1053–1063 (2011).

CAS PubMed PubMed Central Google Scholar

180. 180.

Olagnier, D. et al. Nrf2 negatively regulates STING indicating a link between antiviral sensing and metabolic reprogramming. *Nat. Commun.* **9**, 3506 (2018).

PubMed PubMed Central Google Scholar

#### Download references

#### Acknowledgements

S.R.P. is funded by the European Research Council (ERC-AdG ENVISION; 786602), the Novo Nordisk Foundation (NNF18OC0030274) and the Lundbeck Foundation (R198-2015-171 and R268-2016-3927). T.P. is funded by the European Research Council (ERC-StG IDEM; 637647). S.L.M. acknowledges funding from a Howard Hughes Medical Institute– Wellcome International Research Scholarship and the Sylvia and Charles Viertel Foundation. T.H.M. received funding from Aarhus University Research Foundation (AUFF-E-215-FLS-8-66), the Danish Council for Independent Research-Medical Sciences (4004-00047B) and the Lundbeck Foundation (R268-2016-3927). The authors thank D. Olagnier for critical reading of the manuscript and comments and suggestions.

#### **Author information**

#### Affiliations

1. Department of Biomedicine, Aarhus University, Aarhus, Denmark

Søren R. Paludan & Trine H. Mogensen

2. Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Søren R. Paludan

3. CNRS UMR 5164 ImmunoConcept, University of Bordeaux, Bordeaux, France

Thomas Pradeu

4. Department of Biological and Medical Sciences, University of Bordeaux, Bordeaux, France

Thomas Pradeu

5. Inflammation Division, The Walter and Eliza Hall Institute, Melbourne, VIC, Australia

Seth L. Masters

6. Department of Medical Biology, The University of Melbourne, Melbourne, VIC, Australia

Seth L. Masters

7. Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

Trine H. Mogensen

8. Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark

Trine H. Mogensen

#### Contributions

S.R.P. conceived the idea and wrote the first version of the manuscript together with T.H.M. All authors together fully developed the work, and drafted, finalized and revised the manuscript.

#### **Corresponding author**

Correspondence to Søren R. Paludan.

#### **Ethics declarations**

#### **Competing interests**

The authors declare no competing interests.

#### Additional information

#### Peer review information

*Nature Reviews Immunology* thanks the anonymous reviewer(s) for their contribution to the peer review of this work.

#### **Publisher's note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

#### Glossary

Pattern recognition receptors

(PRRs). A family of germline-encoded immune receptors, including the Toll-like receptors, that detect immunostimulatory molecules to activate signal transduction and gene expression, which induces antimicrobial and inflammatory responses.

#### Constitutive immune mechanisms

Host mechanisms that are constitutively present in an active or latent form and thus can exert host defence activities immediately, independently of inducible processes.

#### Inducible mechanisms

Biological processes that depend on the activation of transcriptional programmes and hence require intermediate steps between the trigger stimulus and effector function.

#### Supramolecular organizing centres

Location-specific higher-order signalling complexes, such as the myddosome in Tolllike receptor signalling, that amplify pattern recognition receptor signalling when pathogen-associated molecular pattern levels exceed specific threshold concentrations.

#### RNA interference

(RNAi). The use of double-stranded RNA molecules containing sequences that match a given gene to knock down the expression of that gene by inhibiting translation of the targeted mRNA or by directing RNA-degrading enzymes to destroy the encoded mRNA transcript.

#### Nuclear domain 10 bodies

(ND10 bodies). Membraneless, interchromatin structures in the nucleus of eukaryotic cells. ND10 bodies are made up mainly of proteins and have been described to be involved in a broad range of processes, including gene regulation, cell cycle, apoptosis, DNA repair and antiviral defence.

#### Aerobic glycolysis

The process by which glucose is converted to lactate in the presence of oxygen to produce energy in the form of ATP.

#### cGAS–STING pathway

(Cyclic GMP–AMP synthase–stimulator of interferon genes pathway). cGAS is a cytosolic DNA-sensing pattern recognition receptor that signals via STING to induce the expression of type I interferon and inflammatory cytokines.

#### RIG-I–MAVS pathway

(Retinoic acid-inducible gene I protein–mitochondrial antiviral signalling protein pathway). RIG-I is a cytosolic RNA-sensing pattern recognition receptor that signals via MAVS to induce the expression of type I interferon and inflammatory cytokines.

#### DNA damage response

Cellular response to DNA damage, including the re-establishment of genome integrity and cell death responses.

#### NLRP3 inflammasome

The NLRP3 inflammasome is activated by danger-associated molecular patterns and molecular signatures associated with homeostasis-altering molecular processes to execute caspase 1-mediated cleavage of molecules such as pro-IL-1 $\beta$  and gasdermin D.

#### NRF2-KEAP1

Nuclear factor erythroid 2-related factor 2 (NRF2) senses oxidative stress, whereupon it is released from Kelch-like ECH-associated protein 1 (KEAP1) to translocate to the nucleus and induce gene expression.

#### Hypoxia-inducible factor $1\alpha$

A transcription factor that is activated by hypoxia to induce the expression of genes with hypoxia-responsive elements in their promoters.

#### Bone morphogenetic protein-SMAD

Bone morphogenetic proteins are growth factors that signal through SMAD proteins to induce gene transcription.

#### About this article

#### Cite this article

Paludan, S.R., Pradeu, T., Masters, S.L. *et al.* Constitutive immune mechanisms: mediators of host defence and immune regulation. *Nat Rev Immunol* (2020). https://doi.org/10.1038/s41577-020-0391-5

### Download citation

- Accepted: 01 July 2020
  Published: 11 August 2020
  DOI: <u>https://doi.org/10.1038/s41577-020-0391-5</u>